

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

Yoshihiro OHMIYA, et al.

Serial No.: 10/526,569 Art Unit: 1652

Filed: March 4, 2005 Examiner: Hope Robinson

For: Secretory or Membrane-binding chimeric protein

DECLARATION

Honorable Commissioner of Patents and Trademarks

Washington, D. C. 20231

SIR:

I, Yoshihiro OHMIYA, declare that:

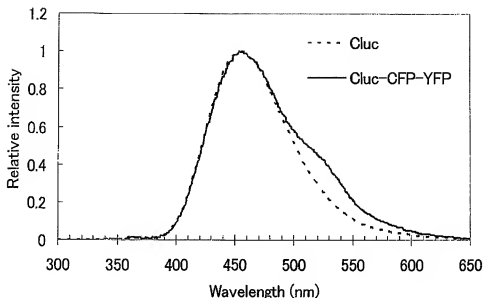
- 1) I am one of the inventors of the above-identified application, and am familiar with the subject matter of said application as well as the disclosures in the cited reference.
- 2) I would like to submit an additional experiment which indicates that an energy transfer occurs between Cypridina luciferase and YFP as it occurs between Vargula luciferase and YFP.

ADDITIONAL EXPERIMENT

A mutant cyan fluorescent protein (ECFP) gene (Clontech, PT3258-5) and a mutant yellow fluorescent protein (EYFP) gene (Clontech, PT3175) were linked to the downstream of a secretory type luciferase

gene derived from *Vargula hilgendorffii* related species *Cypridina noctiluca* (hereinafter abbreviated as "Cluc"; ATTO Corporation, NCBI No. AB177531) to gain a gene construct (SEQ ID NO.1). The gene construct was then inserted into the downstream of CMV promoter to gain pCluc-CFP-YFP vector. The amino acid sequence of the luminescent/fluorescent fusion protein, pCluc-CFP-YFP (116.6 kDa), produced by the said gene is shown in SEQ ID No.2 below. The pCluc-CFP-YFP vector (500 ng) was transfected to NIH3T3 cell using Lipofectamine Plus Reagent. The cell was cultured in DMEM + 10% FBS for 24 hours and then the culture medium was collected. *Vargula* luciferin (1 mM *Vargula* luciferin/ 60 mM phosphate (pH6.4), 20 mM sodium sulfite, 0.3 M sodium ascorbate) was added thereto and the luminescence spectra were measured by LumiFru Spectro Capture AB-1850 (ATTO Corporation).

The result is shown in Fig. 1 below. The light-emitting enzyme alone (Cluc) showed blue light emitting having a peak at 460 nm. On the other hand, the fusion protein had a peak at 530 nm due to the energy transfer from Cluc to YFP. Therefore, it is proved that a luciferase derived from *Vargula* related species *Cypridina noctiluca* can be used as an energy-generating protein of the present invention.



(SEQ ID: No.1)

ATGAAGACCTTAATTCCTTGCCGTTGCATTAGTCTACTGCGCCACTGTTCAATGCCAGGACTGTC
 CTTACGAACCTGATCCACCAACACAGTTCACAACTTCTGTGAAGCTAAAGAAGGAGAATGTAT
 TGATAGCAGCTGTGGCACCTGCACGAGAGACATACTATCAGATGGACTGTGTGAAAAATAAACCA
 GGAAAAACATGTTGCCGAATGTGTCAATGTGAATTGAATGCAGAGTAGAGGCCGAGGATGGT
 TTAGAACATCTATGGAAGAGATTCAGTTCAGGAACCTGGTACATACGTGTGGGTCAAGG
 AACCAAGGGCGGCGACTGGAAGGTGCCATCACCCCTGGAGAACCTGGATGGAACCAAGGGGGCT
 GTGCTGACCAAGACAAGACTGGAAGTGGCTGGAGACATCATTGACATCGCTCAAGCTACTGAGA
 ATCCCATCACTGTAACCGTGGAGCTGACCCCTATCATCGCCAACCCGTACACCATCGGCGAGGT
 CACCATCGCTGTTGTTGAGATGCCAGGCTTCAACATCACCGTCATTGAGTTCTTCAAACTGATC
 GTGATCGACATCTCGGAGGAAGATCTGTAAGAATCGCCCGACACAGCAAAACAAAGGAATGA
 TCTCTGGCCTCTGTGGAGATCTTAAATGATGGAAGATACAGACTTCACCTTCAGATCCAGAACA
 ACTCGCTATTGAGCCTAAGATCAACCAGGAGTTTGACGGTTGTCACCTCTATGGAAATCCTGAT
 GACGTTGCATACGTCAAAGGTCTTCTGGAGCCGTACAAGGACAGCTGCCGCAACCCCATCAACT
 TCTACTACTACACCATCTCCTGCGCCTTCGCCCGCTGTATGGGTGGAGACGAGCGAGCCTCACA
 CGTGCTGCTTGACTACAGGAGACGTCGCGTGTCTCCGAAACTAGAGGAACCTGCGTTTGTCT
 GGACATACTTTCTACGATACATTTGACAAAGCAAGATACCAATTCAGGGTCCCTGCAAGGAGA
 TTCTTATGGCCGCCGACTGTTTCTGGAACACTTGGGATGTGAAGGTTTACACAGGAATGTTGA
 CTCTTACACTGAAGTAGAGAAAGTACGAATCAGGAAACAATCGACTGTAGTAGAAGTCTTGT
 GATGGAACAACAGATTCTGGTTGGAGGAGAAGCGGTGCCGTCCTACAGCTCTCAGAACACTT
 CCATCTACTGGCAAGATGGTGACATACTGACTACAGCCATCCTACCTGAAGCTCTGGTGGTCAA

GTTCAACTTCAAGCAACTGCTCGTACATATTAGAGATCCATTTCGATGGTAAGACTTGCCTT
 ATTTGCGGTAACACTACAACAGGATTTCAAGTATGATTCTTTTGTATGCTGAAGGAGCCTGTGATC
 TGACCCCCAACCCACCGGGATGCACCGAAGAACAGAAACCTGAAGCTGAACGACTCTGCAATAG
 TCTCTTCGCCGGTCAAGATGATCTTGATCAGAAATGTAACTGTGCCACAAGCCTGACCGTGTG
 GAACGATGCATGTACGAGTATTGCCCTGAGGGGACAAACAGGGTTTCTGTGACCACGCATGGGAGT
 TCAAGAAAGAAATGCTACATAAAGCATGGAGACACCTAGAAAGTACCAGATGAATGCAAAaaggg
 caattctcgagatatccagTGGTGAGCAAGGGCGAGGAGCTGTTACCGGGGTGTTGCCCATC
 CTGGTCGAGCTGGACGGCGACGTAAACGGGCCACAAGTTACGCGTGTCCGGCAGGGGCGAGGGCG
 ATGCCACCTACGGCAAGCTGACCTGAAGTTTCATCTGCACCACCGGCAAGCTGCCCGTGCCCTG
 GCCCACCTCGTGACCACCTGACCTGGGGCGTGCAAGTCTTACGCCGCTACCCCGACACATG
 AAGCAGCAGCACTTCTTCAAGTCCGCCATGCCCGAAGGCTACGTCCAGGAGCGCACCATCTTCT
 TCAAGGAGCAGCGCAACTACAAGACCCGCGCGAGGTTGAAGTTCGAGGGCGACACCTTGGTGAA
 CCGCATCGAGCTGAAGGGCATCGACTTCAAGGAGGACGGCAACATCTTGGGGCACAAGCTGGAG
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 ACTTCAAGATCCGCCACAACATCGAGGACGGCAGCGTGCAGCTGCCCGACCACTACCAGCAGAA
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 CTGAGCAAGAGCCCCAACAGAGAGCGCGATCACATGGTCTGCTGGAGTTCTGTGACCCGCGCGG
 GGATCACTCTCGGATCGGACGAGCTGTACAAGgataccagTGGTGAGCAAGGGCGAGGAGCT
 GTTACCGGGGTGGTGCCCATCTGCTGAGCTGAGCAGGCGACGTAAACGGGCCACAAGTTCAAGC
 GTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGCAAGCTGACCTGAAGTTTCATCTGCACCA
 CGGGCAAGCTGCCCGTGCCCTGGCCACCTCGTGACCACTTCGGCTACGGCTGCAAGTGTCT
 CGCCCGCTACCCCGACCATGAAGCAGCAGCACTTCTTCAAGTCCGCCATGCCCGAAGGCTAC
 GTCAGGAGCGCACCATCTTCTTCAAGGACGACGGCACTACAAGACCCGCGCGAGGTGAAGT
 TCGAGGGCGACACCTTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAGGAGGACGGCAA
 CATCTTGGGGCACAAGCTGGAGTACAACATACAAGCCACAACGTCATATCATGGCCGACAAG
 CAGAAGAACGGCATCAAGGTGAACCTTCAAGATCCGCCACAACATCGAGGACGGCAGCTGCGAGC
 TCGCCGACCACTACCAAGCAGAACACCCCATCGGGCAGCGCCCCGTGCTGTGCCCGACAACCA
 CTACCTGAGCTACCAGCTCCGCCCTGAGCAAAGACCCCAACGAGAAGCGCGATCATCATGGTCTGT
 CTGGAGTTCTGTGACCCGCGCGGGATCACTCTGGCATGGACGAGCTGTACAAGctcgag

(SEQ ID: No.2)

M K T L I L A V A L V Y C A T V H C Q D
 C P Y E P D P P N T V P T S C E A K E G
 E C I D S S C G T C T R D I L S D G L C
 E N K P G K T C C R M C Q Y V I E C R V
 E A A G W F R T F Y G K R F Q F Q E P G
 T Y V L G G G T K G G D W K V S I T L E
 N L D G T K G A V L T K T R L E V A G D
 I I D I A Q A T E N P I T V N G G A D P
 I I A N P Y T I G E V T I A V V E M P G
 F N I T V I E F F K L I V I D I L G G R
 S V R I A P D T A N K G M I S G L C G D
 L K M M E D T D F T S D P E Q L A I Q P
 K I N Q E F D G C P L Y G N P D D V A Y

C K G L E P Y K D S C R N P I N F Y Y
 Y L D T S C A F A R C M D E R A S V S
 L T Y R E T A A A P E T R G T C V L
 G I D Y F D T F C A A P E T R G T C V L
 E I L M A D A D F W N T R Q F V K C K
 N V D S Y A T E K I L V G G E A V S T V V
 E L I V D G S I W F K Q D I L T T A I L
 S S A L V T K C G I C G N Y N P P G S D L D
 P E A L V T K C G I C G N Y N P P G S D L D
 S F D A E G A C C L D T P N P P G S D L D
 Q K P E A E R L C N S L F A G C M Y E Y C
 Q K G C N V G H K C P D H A W E F K K N S A D I
 L R G Q Q T L E E E L F T S V S G E G V I L V E L
 K H G D D T L E E E L F T S V S G E G V I L V E L
 P V V S K N G H K F I C V T C F G S R Y P P D H M
 D G D V N G H K F I C V T C F G S R Y P P D H M
 Y G K L T L T K F I C V T C F G S R Y P P D H M
 T L V D D F K N Y K I G S I R H N I E D V L K Q K
 K Q H D D F K N Y K I G S I R H N I E D V L K Q K
 F F K R R I E L Y I S I R H N I E D V L K Q K
 L V N R I E L Y I S I R H N I E D V L K Q K
 H K L E Y N F K I R H N I E D V L K Q K
 N G I K A N F K I R H N I E D V L K Q K
 A D H Y S Q N T P I S G I T L G M D P D N M
 H Y L L S F V T A A G I T L G M D P D N M
 V L L E F V S T A A G I T L G M D P D N M
 D I P V V S K N G H K F I C V T C F G S R Y P P D H M
 E L D V G D V S K N G H K F I C V T C F G S R Y P P D H M
 A T Y G K V T T F F K Y S A M T R A E V E D F E R
 W P T L V H D D F K Y S A M T R A E V E D F E R
 H M I K F F D D F K Y S A M T R A E V E D F E R
 T I L V N R I E L Y I S I R H N I E D V L K Q K
 D L G K N L E Y N F K I R H N I E D V L K Q K
 Q K L A D H Y S F V T A A G I T L G M D P D N M
 L A H Y L L E Y N F K I R H N I E D V L K Q K
 D N M V L L E Y N F K I R H N I E D V L K Q K
 Y K L E

I, the undersigned, declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: Nov. 27th, 2007

Y. C.
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